S U M M A R Y

RESEARCH ON THE EFFECTS OF BEHAVIOURAL REACTIVITY ON PRODUCTION OUTPUTS AND REPRODUCTION EFFICIENCY IN BOVINES

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The present Doctoral Thesis is structured in two parts. ***Part I*** *consists of the bibliographic study, which encompasses three chapters and spans over 44 pages, as follows:*

Chapter 1

PRINCIPLES OF BOVINES BIOLOGY AND ETHOLOGY, presents notions of cattle ethology, highlighting the importance of this discipline in studying farm animal behaviour and enhancing their welfare. It describes aspects such as behavioural reactivity, temperament, and the influence of genetic factors on behaviour, emphasizing the differences between cattle and water-buffaloes. This chapter also discusses the anatomical and physiological differences between these two species, which influence their adaptability to different environments and production systems, highlighting the importance of understanding these aspects for optimizing production and maintaining animal health. It also addresses the economic and ecological implications of bovines farming, emphasizing the need to balance productivity and sustainability.

Chapter 2

MAIN BEHAVIORAL PATTERNS IN BOVINES, explores the main behavioural patterns in bovines, including specific behaviours such as rumination, resting, nutrition, communication, behaviour during thermal stress, social, reproductive, aggressive, and learning behaviour. It provides details about the mechanism of rumination and its importance for digestion, resting behaviour and its impact on milk production, and feeding behaviour, highlighting the influence of various factors on these behaviours. This chapter also discusses agonistic and play behaviours, dam-calf interaction, and behaviour during calving. It further explores stereotypical behaviours, urination and

defecation patterns, offering an overview of the diversity of bovines behaviours and their impact on welfare.

Chapter 3

BEHAVIOURAL TESTS USED IN ETHOLOGY STUDIES IN BOVINES, explores various methods and tests used in bovine ethology to quantify temperament and specific behaviours. The main methods for assessing temperament include subjective evaluations, natural behaviour coding, and experimental observations. These methods help identify temperamental traits such as shyness-boldness, activity, aggressiveness, and sociability. Common behavioural tests presented in this chapter include milking temperament, "crush" test on the weighing platform, human approach tests, and social isolation tests. It also describes precision livestock farming (PLF) tools, such as accelerometers, video cameras, and microphones, which allow real-time data collection for optimizing farm management. This chapter briefly describes the use of these technologies, which facilitate the early detection of health issues, optimize nutrition, and monitor the level of welfare, contributing to a sustainable and efficient dairy production.

The second part of the thesis *is structured into four chapters, along with the general conclusions and references, spanning on 173 pages, as follows:*

Chapter 4

This chapter outlines the **study's aim and objectives**, **materials and methods** and **statistical analysis**.

The aim of this current thesis was to evaluate the impact of behavioural reactivity (temperament) on production and reproduction efficiency and welfare in dairy cattle and water-buffaloes.

The general objectives were:

- To evaluate the effects of temperament on production efficiency in cattle and water-buffaloes;
- To determine the impact of behavioural reactivity on reproduction indices in cattle and water-buffaloes;
- To study the implications of dairy cattle and water-buffaloes temperament on health and welfare.

The present work comprised a set of six distinct experiments, with a biological material consisting of 938 bovines; of which 776 were dairy-cattle and 162 were water-buffaloes.

The research was conducted in the experimental farms of the Research and Development Institute for Bovine - Balotesti, University of Wisconsin - Madison, USA, Research and Development Station for Bovine - Arad, Research and Development Station for Water-Buffaloes - Şercaia, and one commercial farm, Transilvania Dairy Farm - Mesendorf.

1st Experiment: Milking temperament in Romanian Black and White Spotted dairy cows, raised under tied stall housing system

This experiment was conducted at the Research and Development Institute for Bovine Balotesti on 91 Romanian Black and White Spotted dairy cows, from May to July 2020. Cows were housed in two identical tied barns with short stalls, arranged in two rows "head-to-head," using straw as bedding, with ad libitum access to water and mineral blocks. The cows' daily ration included 35 kg of corn silage, 6 kg of alfalfa hay and 6 kg of concentrates. Milking was done twice daily in the barn using individual milking machines. Temperament at milking was assessed using a scale from 1 to 5, with cows classified as "calm" or "nervous". Production, reproduction, and welfare indicators were collected, including milk production/standard lactation (305 days), milking speed, body condition score (BCS), cleanliness of udder, rump, and hind legs, body weight, number of artificial inseminations per gestation, age at first calving, calving interval, incidence of mastitis and retained placenta, incidence of tarsal joint lesions, and number of oocysts per gram of feces (OPG). The data were statistically analysed to estimate the effects of milking temperament on production, reproduction, and welfare indicators. Moreover, a subset of 34 cows was selected, classified into two groups based on the presence or absence of horns (n=17 for each group), to further investigate the effects of this factor on productive and reproductive efficiency.

2nd Experiment: Milking temperament in American Holstein dairy cows, raised under loose housing system

Conducted at Emmons Blaine Dairy Cattle Center in Wisconsin, USA, from October 2023 to May 2024, this study included a number of 439 Holstein dairy cows. Milking temperament was evaluated using a scale (0–2), analysing cow's behaviour during three time points, as follows udder preparation for milking, teat cup attachment, and during milking, with animals being classified in 3 groups as follows: calm, moderate, and nervous cows. The cows were housed in a semi-open barn with loose system, without access to paddocks, with a linear feeding area. The animals were housed in individual sand-bedded stalls, arranged in 4 rows "head-to-head," with ad libitum access to water and mineral blocks. The same diet, a total mixed ration (TMR), was offered ad libitum to all cows. The cows were fed three times a day at 09:00, 15:00, and 21:00. Milking was performed twice daily at 03:00 AM and 15:00 PM in a 2x16parlour. Milk production/standard lactation (305 days), milking speed at the day of temperamental evaluation, calving interval, number of inseminations per gestation, somatic cell count, fat and protein percentages were statistically analysed to estimate the effects of milking temperament on production, reproduction, and welfare indicators.

3rd Experiment: Milking temperament in Romanian Spotted dual-purpose cows, raised under loose housing system

This study was conducted at the Research and Development Station for Bovine Arad, on a number of 149 Romanian Spotted dual-purpose cows, from July to August 2023. The cows were housed in free barns and milked in a 2x14 "herring-bone" parlour. The cows were housed on deep straw bedding, with an allocated resting area of 9 m^2, with ad libitum access to water in outdoor paddocks. The daily ration consisted of 15 kg of green fodder, 12 kg of corn silage, 6 kg of alfalfa hay, and 6 kg of concentrates from spring to late autumn, and a ration of 25 kg of corn silage, 6 kg of alfalfa hay, and 6 kg of concentrates during winter. The animals were fed twice daily in the paddock, with a feeding space of 70-75 cm per animal. Temperament at milking was assessed using a scale from 1 to 5, with cows classified as "calm" or "nervous." Data on production, reproduction and welfare indicators were collected to evaluate the effects that milking temperament has on these variables: body condition score (BCS), hock lesions incidence, cleanliness of the udder, excessive hoof growth, milk yield (kg/305 days of lactation), fat and protein production, calving interval, dry period duration, gestation duration, age at first calving, dry period, number of inseminations per gestation, and somatic cell count.

4th Experiment: Temperament during weighing in Black and White Spotted Romanian dairy cows, raised under tied stall housing system

This study was conducted at the Research and Development Institute for Bovine Balotesti and included a number of 97 cows, conducted over two consecutive years (September 2021 and September 2022). Temperament assessment was carried out during weighing (chute score), using a scale from 1 to 4. Moreover, the manner of exiting post chute score was separately evaluated. Production, reproduction, welfare, and physiological indicators were collected to determine the impact of temperament on the following variables: body weight, body condition score (BCS), cleanliness of udder, rump, and hind legs, tarsal joint lesions, number of inseminations per gestation, age at first calving, calving interval, somatic cell count, fat, protein, lactose, solids, urea, casein content, milk density, and pH, infrared thermography data, and blood cortisol levels.

5th Experiment: Milking temperament in Romanian Dairy Water-Buffaloes raised under tied stall housing system

This experiment took place at the Research and Development Station for Water-Buffaloes Sercaia, on 60 water-buffalo cows, from May till August 2022. The animals had access to natural pasture for 10-12 hours/day, without supplementation of the diet with hay or concentrates during summer season. During night, buffalo cows were housed in a tied barn, using straw bedding, with ad libitum access to water and mineral blocks. The buffaloes were milked twice daily in the barn, using individual milking machines. Milking temperament was evaluated by two observers using a scale from 1 to 5, with animals classified as "calm" or "nervous". Data on production, reproduction, and welfare indicators were collected, including milk yield per first 100 days in milk (DIM), milking

speed, body condition score (BCS), cleanliness of udder, rump, and hind legs, tarsal joint lesion, skin lesions, excessive hoof growth, hairless patches, nasal, ocular, and vulvar discharges incidence, age at first calving, calving interval, and infrared thermography data. The data were statistically analysed to estimate the effects of milking temperament on production, reproduction, and welfare indicators in water buffaloes raised in an extensive system.

6th Experiment: Milking temperament in Romanian Dairy Water-Buffaloes raised under loose housing system.

The research was conducted at Transylvania Lactate farm, on 102 water-buffalo cows, from May till August 2022. Milking was done twice daily, in a 2x8 "herring-bone" parlour. The buffalo cows were housed in free barns with individual resting stalls, with an allocated space of 10 m^2 in the movement area, with ad libitum access to water, mineral blocks, and outdoor paddocks. The daily ration consisted of 7 kg alfalfa hay, 7 kg pasture hay, 4 kg corn silage, and 2.5 kg concentrates, administered in two feedings, as total mixed ration (TMR). The cows were evaluated during milking using a scale from 1 to 5, then classified into calm and nervous temperament groups. Data were collected, including milk yield per 100 days in milk, milking speed, body condition score (BCS), cleanliness of udder, rump and hind legs, excessive hoof growth, skin lesions incidence, hairless patches, nasal, ocular and vulvar discharges incidence, tarsal joint lesions, age at first calving, and calving interval. The data were statistically analysed to estimate the effects that milking temperament has on production, reproduction, and welfare indicators in water buffaloes raised in an intensive system.

The statistical approach included running statistical tests and data visualization methods to analyse data regarding the temperament of cows and water-buffaloes and effects on production, reproduction, and welfare indicators. Descriptive statistics were calculated for each variable, and normality tests were applied to determine the data distribution.

Based on these results, parametric or non-parametric tests were used, such as Student's t-test, ANOVA, Mann-Whitney U test, and Spearman's correlation. Linear regressions and principal component analysis (PCA) were also applied to examine the relationships between variables.

Data were visualized using various graphic techniques, including box plots, scatter plots, and heatmaps, to facilitate interpretation and study conclusions.

All statistical analyses were performed using specialized software (Microsoft Excel, Minitab17 (Minitab LLC®), Minitab Workspace, and R® software), with a significance level set at $p \leq 0.05$.

Chapter 5

This chapter presents the **results and discussions** for each experiment, describing the impact of temperament on production, reproduction, and welfare indicators in dairy cattle and water-buffaloes.

1st Experiment - Effects of behavioural reactivity during milking and effects of horns on production and reproduction efficiency, and welfare indicators in dairy cows raised under tied stall housing system:

- Behavioural reactivity during milking significantly influenced the milk yield per standard lactation ($p \leq 0.05$) with calm cows having a higher milk yield compared to nervous cows. Calm cows had an average milk yield of 8116.65 kg per lactation, while the nervous cows recorded an average yield of 7401.19 kg per lactation;

- The relationship between body condition score (BCS) and cow's temperament at milking was significant ($p \leq 0.05$); calm cows had a better body condition and higher fat deposits compared to nervous cows throughout the lactation;

- Reproductive indicators were not significantly influenced ($p > 0.05$) by the cows milking temperament;

- Welfare indicators, such as tarsal joint lesions, cleanliness of rump, udder, and hind legs, were not influenced ($p > 0.05$) by the behavioural reactivity during milking;

- Health indicators, such as mastitis and retained placenta incidence, were not statistically influenced ($p > 0.05$) by the cow's behavioural reactivity during milking.

2nd Experiment - Effects of behavioural reactivity during milking on production, reproduction efficiency and welfare in dairy cows raised under loose housing system:

- The temperament of cows during udder preparation for milking significantly influenced ($p \leq 0.05$) milk yield, with calm cows having a higher milk yield compared to moderate-reactive and nervous cows. The average milk yield/standard lactation was 7591.00 kg across the entire studied population, with calm cows having an average yield of 8779.00 kg, moderate-reactive cows having an average yield of 7293.00 kg, and nervous cows having an average yield of 7482.00 kg;

- Behavioural reactivity during teat cup attachment phase significantly influenced ($p \leq 0.05$) somatic cell count, with nervous cows having a higher somatic cell count in milk compared to calm cows. Calm cows had an average of 107.00 thousand cells/mL, moderate-reactive cows had 132.40 thousand cells/mL, and nervous cows had 202.60 thousand cells/mL;

- Behavioural reactivity during milking significantly influenced ($p \leq 0.05$) milk yield, with moderate-reactive temperamental cows having higher milk yields compared to calm cows. Calm cows had an average yield of 6887.00 kg, moderate cows had an average yield of 7969.00 kg, and nervous cows had an average yield of 7443.00 kg. The temperament during milking significantly influenced ($p \leq 0.05$) the somatic cell count, with moderate cows having a higher somatic cell count in milk compared to calm cows.

Calm cows had an average of 50.10 thousand cells/mL, moderate cows had 148.50 thousand cells/mL, and nervous cows had 147.60 thousand cells/mL;

- No significant differences ($p>0.05$) were observed between temperament classes and variables such as milking speed, calving interval, number of artificial inseminations per gestation and milk fat percentage.

3rd Experiment – Effects of behavioural reactivity during milking on production, reproduction efficiency and welfare in dual-purpose cows raised under loose housing system:

- Milking temperament significantly influenced ($p\leq0.05$) gestation length, with calm cows during milking having a shorter gestation length, compared to nervous cows;
- Behavioural reactivity during milking significantly influenced ($p\leq0.05$) the cleanliness of the udder, with calm cows having a lower percentage of dirty udders;
- Regarding milk production per standard lactation, parameters reflecting milk quality such as fat and protein content and somatic cell count in milk, no significant differences ($p>0.05$) were found between the two groups of calm and nervous cows.

4th Experiment – Effects of behavioural reactivity during weighing on production, reproduction efficiency and welfare in dairy cows raised under tied stall housing system:

- The temperament of cows during the time spent in the weighing scale, moderately influenced ($p\leq0.05$) milk quality parameters, especially lactose, protein and solids percentages. At the same time, the temperament of cows at the exit from the weighing platform (exit velocity score) influenced ($p\leq0.05$) milk quality, particularly milk density;
- Nervous cows had higher serum cortisol levels ($p\leq0.05$) compared to calm cows, as well as significant differences ($p\leq0.05$) in infrared temperature measured at regio orbitalis and regio nasalis;
- Nervous animals showed a more pronounced decrease in temperament scores over time, compared to calm animals, with a potential cause being represented by habituation to the handling system;
- No associations ($p>0.05$) were found between temperament scores and body weight, milk yield, body condition score, calving interval, or the number of inseminations per gestation;
- Although there were some variations in the coefficients and odds ratios associated with temperament during weighing and chute exit velocity, their influence on animal welfare indicators was not statistically significant ($p>0.05$).

5th Experiment – Effects of behavioural reactivity during milking on production, reproduction efficiency and welfare in water-buffaloes raised under tied stall housing system:

- Milking temperament significantly influenced (p≤0.01) the amount of milk produced in the first 100 days of lactation, with calm water-buffaloes having a higher milk yield compared to nervous water-buffalo cows. The average milk yield in the first 100 days of in milk was 828.80 kg for the entire studied population, 924.32 kg for calm water-buffalo cows, and 593.02 kg for nervous water-buffalo cows;

- Behavioural reactivity during milking significantly influenced (p≤0.001) the milking speed, with calm temperament water-buffaloes having a higher milk ejection rate. The average milking speed for the entire study population was 0.48 kg milk/min, calm buffalo cows had a milking speed of 0.58 kg/min, while nervous buffaloes had an average milking speed of 0.26 kg/min;

- Regarding reproductive indicators, water-buffalo cows milking temperament did not had a statistical influence (p>0.05);

- Regarding animal welfare indicators, temperament at milking did not significantly influence (p>0.05) variables such as body condition score, excessive hoof growth, skin lesions, hairless patches, tarsal joint lesion, cleanliness of rump, udder and hind legs, ocular, nasal, and vulvar discharges incidence;

- Regarding infrared thermography, the temperature captured before and after milking was not influenced by the milking temperament (p>0.05), with calm buffaloes showing an average decrease of 0.15°C in muzzle temperature after milking, while nervous buffalo cows had an average increase of 0.84°C.

6th Experiment – Effects of behavioural reactivity during milking on production, reproduction efficiency and welfare in water-buffalo cows raised under loose housing system:

- Milking temperament significantly influenced (p≤0.05) the amount of milk produced per 100 days of lactation, with calm buffalo cows having higher milk yields compared to nervous buffalo cows. The average milk yield per 100 days of lactation was 378.03±21.681 kg for the entire studied population, 399.20±24.244 kg milk for calm buffalo cows, and 316.15±45.717 kg milk for nervous water-buffalo cows;

- Behavioural reactivity during milking significantly influenced (p≤0.05) the milking speed;

- Regarding reproductive indicators, water-buffalo temperament at milking did not had a statistical influence (p>0.05);

- Regarding animal welfare indicators, water-buffaloes milking temperament did not significantly influence (p>0.05) variables such as body condition score, excessive hoof growth, skin lesions, hairless patches, tarsal joint lesions, cleanliness of rump, udder and hind leg, ocular, nasal and vulvar discharges incidence.

Chapter 6

This chapter presents the novelty of the current Doctoral Thesis. The **originality** of the work was expressed through the evaluation of temperament in dairy cattle and water-buffaloes, including farms practicing extensive and/or intensive rearing systems, predominantly studying local breeds (Romanian Black and White Spotted, Romanian Spotted, and Romanian Water-Buffalo), integrating complex approaches related to production, reproduction efficiency, animal health, precision livestock farming, endocrinology, behaviour and animal welfare.

Chapter 7

This chapter consists of the **final conclusions and recommendations** of the Doctoral Thesis.

References

This section includes a total of 272 bibliographic references.